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Synthesis and Antimicrobial Evaluation of Potent N-Hydroxy-2,4-diaryl-3-azabicyclo[3.3.1]nonan-9-ones and Their Oximes

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N-Hydroxy-2,4-diaryl-3-azabicyclo[3.3.1]nonan-9-ones [**2a-2c**] obtained by the reaction between the 2,4-diaryl-3-azabicyclo[3.3.1]nonan-9-ones [**1a-1c**] and *m*-chloroperbenzoic acid, then N-hydroxy-2,4-diaryl-3-azabicyclo[3.3.1]nonan-9-one oximes [**3a-3c**] obtained by the reaction between N-hydroxy-2,4-diaryl-3-azabicyclo[3.3.1]nonan-9-one and hydroxyl amine hydrochloride are screened against selected bacteria (*Vibro cholerae, Salmonella typii, Shigella flexneri, Escherichia coli, Staphylococous aureus, Klebsiella pneummonia, β-Heamolytic streptococcus* and *Pseudomonas*) and fungi (*Aspergillus flavus, Mucor, Microsporum gypseum* and *Rhizopus*). Disc diffusion method is employed to determine the *in vitro* antibiotic effect. The inhibitory effects of the compounds are very close and identical in magnitude and are comparable with that of the standard antibiotic used.

Key Words: Antibacterial, Antifungal, N-Hydroxy-2,4-diaryl-3-azabicyclo[3.3.1]nonan-9-ones, Oximes.

INTRODUCTION

Many hydroxy compounds like 2,4-dihydroxyacetophenones have antibacterial activity[1]. Oximes, semicarbazones, thiosemicarbazones, chalcones *etc.* and their derivatives possess antibacterial[2-4], anticancer[5], antimalarial[6], antiviral[7], antitubercular[8] and antilepral[9] activities. Some hydroxylamines and ketooximes have been reported as effective antibacterial, antifungal and antileukemic agents. For example N-hydroxy urea, one of the effective antineoplasmic agents and cicloproxolamine has broadspectrum antibacterial and antifungal activity. Therefore it has become attractive to synthesize N-hydroxyheterocycles and oximes to determine the *in vitro* potency against test bacteria and fungi. In this paper, we have reported the synthesis (**Scheme-I**) and bactericidal, fungicidal ability of N-hydroxy-2,4-diaryl-3-azabicyclo[3.3.1]nonan-9-ones [**2a-2c**] and N-hydroxy-2,4-diaryl-3-azabicyclo[3.3.1]nonan-9-one oximes [**3a-3c**].

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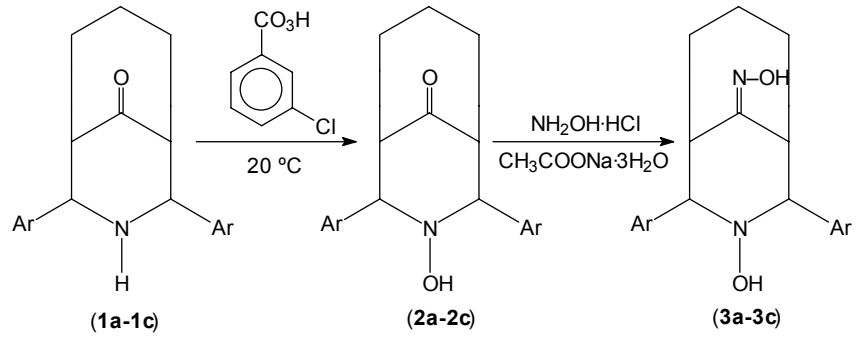

1a, 2a, 3a: Ar = C_6H_5; **1b, 2b, 3b:** Ar = *p*–$OCH_3C_6H_4$; **1c, 2c, 3c:** Ar = *o*-ClC_6H_4

Scheme-I

EXPERIMENTAL

Preparation of media

Nutrient broth was used to cultivate bacteria. Agar media was prepared by adding 24 % w/v agar in the nutrient broth for making agar slants. Bacteria were sub-cultured on the nutrient agar slants. The inoculum was prepared by transfering loopfull of the corresponding organism from the stock culture into the sterile broth and incubated at 37 °C for bacterial stains. 20 mL of sterile nutrient agar media was added to each petri dish and 2 mL of 24 h broth culture of bacteria was then added to the respective plates and mixed thoroughly by rotatory motion of the plates. The respective hydrochloride of **2a-2c** and **3a-3c** were dissolved in water in the concentration of 10 mg/mL. The solution was maintained as a stock solution. The different concentrations (100, 200 and 500 ppm) were prepared from the stock solution. Sterile paper disc of 5 mm diameter was saturated with the three different concentrations and such discs were placed in each seeded agar plates. The petri plates were incubated at 37 °C and zones of inhibitions were measured excluding the diameter of the paper disc (5 mm). Control discs were performed with sterile water.

For the antifungal activity assay, the *in vitro* disc diffusion method was adopted. Sabouraud's Dextrose agar was used to culture the fungi. Peptone water (1 %) was used for fresh culture of all the fungi and was maintained by periodic sub culturing in fresh Sabouraud's Dextrose medium. Plates for Sabouraud's Dextrose medium were prepared with the inocula by adding 1 mL of dilute culture of the test organism. The respective hydrochlorides of **2a-2c** and **3a-3c** were dissolved in water in the concentration of 10 mg/mL. The solution was maintained as a stock solution. The different concentrations (100, 200 and 300 ppm) were prepared from the stock solution. Sterile paper disc of 5 mm diameter was saturated with the three different concentrations and such discs were placed in each seeded agar plates. The

petri plates were incubated at 30 °C for 70 h. The inhibition zones are measured excluding the diameter of the paper disc (5 mm). At 500 mg/mL concentration the conventional standard antifungal drug ketoconazole exhibited 20 ± 0.5 mm zone of inhibition against all the test fungi.

Preparation of N-hydroxy-2,4-diaryl-3-azabicyclo[3.3.1]nonan-9-one oximes [3a-3c]: The respective N-hydroxy-2,4-diaryl-3-azabicyclo-[3.3.1]nonan-9-ones [0.05 mol] and sodium acetate trihydrate (0.15 mol) were dissolved in boiling ethanol and hydroxylamine hydrochloride (0.06 mol) was added. The mixture was heated under reflux for 15 min and poured into water. The separated solid was filtered off and recrystallized from ethanol. The elemental analysis, melting points, yields are given in Table-1. The ^{1}H NMR and ^{13}C NMR chemical shifts are given in Tables 2 and 3.

TABLE-1
PHYSICAL AND ANALYTICAL DATA OF
COMPOUNDS **2a-2c** AND **3a-3c**

Compd.	m.p. (ºC)	Yield (%)	m.f.	Elemental analysis: Found (Calcd.) %		
				C	H	N
2a	192	70	$C_{20}H_{21}NO_2$	78.09 (78.17)	6.50 (6.84)	4.31 (4.56)
2b	238	65	$C_{22}H_{25}NO_4$	70.33 (71.93)	7.16 (6.81)	3.95 (3.81)
2c	210	72	$C_{20}H_{19}NO_2Cl_2$	72.11 (72.30)	7.66 (7.52)	3.28 (3.71)
3a	173	74	$C_{20}H_{22}N_2O_2$	74.29 (74.53)	6.99 (6.83)	8.95 (8.70)
3b	197	70	$C_{22}H_{26}N_2O_4$	70.33 (69.11)	6.24 (6.81)	7.11 (7.33)
3c	188	68	$C_{20}H_{20}N_2O_2Cl_2$	71.29 (71.10)	6.50 (6.72)	7.53 (7.30)

Preparation of N-hydroxy-2,4-diaryl-3-azabicyclo[3.3.1]nonan-9-ones [2a-2c]: The respective 2,4-diaryl-3-azabicyclo[3.3.1]nonan-9-ones and *m*-chloroperbenzoic acid (1:1 mole ratio) were mixed in 20 mL of chloroform at 0 °C. The mixture was shaked well and kept for overnight at 25 °C. Then the mixture was extracted with chloroform and washed with 10 % sodium bicarbonate solution. The chloroform layer was dried with anhydrous sodium sulfate and evaporated. The separated solid was subjected to column chromatography.The elemental analysis, melting point and yield of N-hydroxy-2,4-diaryl-3-azabicyclo[3.3.1]nonan-9-ones are given in Table-1.

TABLE-2
^{1}H NMR CHEMICAL SHIFT VALUES OF **2a-2c** AND **3a-3c**

Proton number	**2a** (δ ppm)	**3a** (δ ppm)	**2b** (δ ppm)	**3b** (δ ppm)	**2c** (δ ppm)	**3c** (δ ppm)
H-5	2.66-2.75	2.52	2.62-2.86	2.81	2.52-2.63	2.63
H-1	2.66-2.75	2.57	2.62-2.86	2.68	2.52-2.63	2.63
H-4	4.38	4.19	4.86	4.93	4.29	4.09
H-2	4.38	4.25	4.86	4.97	4.29	4.12
H-6	1.67-1.74	1.73-1.76	1.70-1.75	1.79	1.59-1.67	1.67
H-8	1.67-1.74	1.78-1.82	1.70-1.75	1.90	1.59-1.67	1.69
H-7	1.97-1.99	1.41-1.55	1.87-1.88	1.52-1.60	2.00-2.11	1.59
N-OH	4.88	4.89	4.98	5.04	4.87	4.82
C=N-OH	-	8.22	-	7.88	-	7.70
Aromatic	7.29-7.48	7.25-7.40	7.28-7.45	7.25-7.37	7.29-7.46	7.26-7.42
p-OCH_3 (2)	-	-	3.95	3.95	-	-
p-OCH_3 (4)	-	-	3.95	3.97	-	-

TABLE-3
^{13}C NMR CHEMICAL SHIFT VALUES OF
COMPOUNDS **2a-2c** AND **3a-3c**

Proton number	**2a** (δ ppm)	**3a** (δ ppm)	**2b** (δ ppm)	**3b** (δ ppm)	**2c** (δ ppm)	**3c** (δ ppm)
C-2	74.15	75.48	71.50	76.40	73.94	76.43
C-4	74.15	73.69	71.50	75.12	73.94	76.43
C-5	54.44	37.64	51.20	55.10	54.69	55.33
C-1	54.44	44.83	51.20	55.22	54.69	55.33
C-6	29.43	26.59	21.50	21.0	21.36	21.76
C-8	29.43	28.21	21.50	26.15	21.36	26.85
C-7	21.20	21.72	29.82	31.25	29.38	30.92
C=N-OH	213.00	163.23	217.60	161.00	216.00	161.00
Ipso	139.76	140.74	131.60	139.10	131.91	139.09
p-OCH_3	-	-	55.41	54.124	-	-
Aromatic	127.13-128.80	127.17-128.67	128.30-129.00	128.15-128.62	128.15-128.30	128.26-129.42

Preparation of 2,4-diaryl-3-azabicyclo[3.3.1]nonan-9-ones [1a-1c]: A mixture of cyclohexanone (10 mL, 0.10 mol), benzaldehyde (21 mL, 0.20 mol), dry ammonium acetate (8 g, 0.10 mol) and 95 % ethanol (50 to 150 mL) was gently warmed on a hot plate till the yellow colour formed during the mixing of the reactants, just turned orange (5-10 min). The mixture was cooled and ether was added to it until a clear orange solution was obtained. It was allowed to stand (1-3 d) till no more crystals separated

out. The pale yellow crystals of 2,4-diary-l-3-azabicyclo[3.3.1]nonan-9-ones were filtered off and washed with a mixture of ethanol and ether (1:1), till the solid becomes almost colourless. The ketone[10] obtained was recrystallised from benzene.

Instrumentation: Proton NMR spectra were recorded on a Bruker AMX-400 spectrometer operating at 100 MHz. Samples were prepared by dissolving about 10 mg of sample in 0.5 mL of $CDCl_3$, containing 1 % TMS. All the chemical shifts are in reference to TMS. ^{13}C NMR spectra were recorded on a Bruker AMX-400 spectrometer operating at 400 MHz and using 10 mm sample tubes. Solution for the measurement of spectra were prepared by dissolving 0.5 g of the sample in 2.5 mL of $CDCl_3$ containing 1 % TMS. All the chemical shifts are in reference to TMS.

RESULTS AND DISCUSSION

Antibacterial activity: The N-hydroxy-2,4-diaryl-3-azabicyclo[3.3.1]-nonan-9-ones [**2a-2c**], N-hydroxy-2,4-diaryl-3-azabicyclo[3.3.1]nonan-9-ones oximes [**3a-3c**] are screened for their bactericidal activity. The method followed for the present investigation is disc diffusion method suggested by Maruzella *et al.*[11]. The bacterial strains used are *Vibro cholerae, Salmonella typii, Shigella flexneri, Escherichia coli, Staphylococous aureus, Klebsiella pneumonia β-Heamolytic streptococcus* and *Pseudomonas*. The *in vitro* inhibition profiles of the compounds are given in Table-4. Each value is an average of three determinations. It is apparent from Table-2 that the compound **2a** is active against all the test bacteria. It is found that the compounds **3a** and **3b** are inactive against *Escherichia coli, Klebsiella pneumonia* and *Pseudomonas*. The inhibitory effects of the compounds are close and identical in magnitude and are comparable with that of the standard antibiotic used. In this study, the conventional standard antibacterial drug chloramphenicol at 500 µg/mL concentration exhibited 30 ± 0.5 mm zone of inhibition against all the test bacteria.

Antifungal activity: The inhibitory effect of the products **2a-2c** and **3a-3c** against select fungi are studied in detail. The method used for this study is disc diffusion method. The fungal stains used in the study *viz.*, *Aspergillus flavus, Mucor, Microsporum gypseum* and *Rhizopus*. The fungal responses are presented in Table-5. Each value is an average of three determinations. From the Table-5 it is known that compounds **2b**, **2c**, **3b** and **3c** are inactive against *Mucor*. The inhibitory effects of the compounds are close and identical in magnitude and are comparable with that of the standard antibiotic used. In this study, the conventional standard antifungal drug ketoconazole at 500 µg/mL concentration exhibited 20 ± 0.5 mm zone of inhibition against all the test fungi.

TABLE-4
in vitro INHIBITION PROFILE OF THE COMPOUNDS **2a-2c** AND **3a-3c** AGAINST TEST BACTERIA

Bacteria	**2a** (ppm)			**2b** (ppm)			**2c** (ppm)			**3a** (ppm)			**3b** (ppm)			**3c** (ppm)		
	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500
S. aureus	10	12	17	7	11	19	7	12	20	12	16	21	7	10	12	9	12	13
Pseudomonas	14	16	21	11	16	19	12	14	19	–	–	–	–	–	–	–	–	–
Klebsiella	7	9	13	9	13	20	8	12	20	–	–	–	–	–	–	6	9	12
E. coli	10	14	20	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
β-H. streptococcus	30	34	38	9	13	16	7	10	18	24	27	31	16	19	24	16	20	22
S. typii	27	31	36	30	34	39	26	33	35	30	36	39	22	25	30	21	24	29
V. cholerae	25	28	31	15	19	26	14	13	25	24	29	35	20	24	29	21	26	30
S. flexneri	10	14	19	14	16	21	14	15	25	22	24	29	–	–	–	–	–	–

All values are in millimeter (mm), representing the diameter of the zone of inhibition, (–) no activity.

TABLE -5
in vitro INHIBITION PROFILE OF THE COMPOUNDS **2a-2c** AND **3a-3c** AGAINST TEST FUNGI

Fungi	**2a** (ppm)			**2b** (ppm)			**2c** (ppm)			**3a** (ppm)			**3b** (ppm)			**3c** (ppm)		
	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500
M. gypseum	17	20	24	21	24	28	20	24	30	20	22	29	24	26	30	25	26	30
A. flavus	18	23	27	19	23	25	18	25	30	18	20	24	17	20	26	16	21	25
Rhizopus	17	21	27	14	19	26	18	20	26	16	20	25	14	18	24	16	20	27
Mucor	20	22	25	–	–	–	–	–	–	17	21	24	–	–	–	–	–	–

All values are in millimeter (mm), representing the diameter of the zone of inhibition, (–) no activity.

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